BIOTECHNOLOGY IN THE PULP AND PAPER INDUSTRY

A report prepared for the National Biotechnology Advisory Committee

by

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FOREWORD

This document was prepared under contract by the Pulp and Paper Research Institute of Canada for Industry, Science and Technology Canada, on behalf of the National Biotechnology Advisory Committee.

The objective of the study was to determine the current status of biotechnology in the pulp and paper industry in Canada, and to provide a basis for public discussion on possible courses of future action in terms of: the awareness of developments in this field and the readiness and prospects for technology transfer; the need for training programs for scientists; the role of industry, as well as universities and government, in promoting biotechnology in the industry; and the regulatory climate.

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EXECUTIVE SUMMARY

This report was commissioned as a discussion paper for the National Biotechnology Advisory Committee. It reviews and critically evaluates progress in biotechnology as applied to the pulp and paper industry since 1984, when the subject was previously treated by the Science Council of Canada in a manuscript report entitled "Biotechnology in the Pulp and Paper Industry". The objective of this present report and subsequent workshop to be organized by the NBAC is to initiate action in Canada to assist industry in implementing those biotechnologies which look most promising.

The first section of the report highlights the most recent advances in biotechnology, divided into those already applied on an industrial scale, those under development (applied research), and fundamental research. Biological waste water treatment has been introduced to many more mills in Canada since 1984, especially kraft mills. This has generally been in response to legislation concerning biological oxygen demand (BOD), toxicity and suspended solids. It is hoped that secondary treatment will also substantially decrease chloroorganic discharges as measured by the AOX parameter. Many mills have chosen activated sludge treatment instead of the more traditional aerated lagoons, due mainly to constraints in land availability. Several mechanical pulp mills have installed pilot and mill-scale anaerobic treatments. These appear to be especially effective in CTMP mills, which have concentrated BOD effluent.

Enzyme treatments to facilitate bleaching of kraft pulp, and to decrease pitch deposition in papermaking have been successful tested abroad on a mill-scale. Another enzyme treatment, with cellulase, increased dewatering rates of recycled furnish during papermaking. In Canada, mill-scale applications of some of these technologies are being

planned for the near future. Advances in applied research were noted in biological bleaching and in biomechanical pulping with fungi. In fundamental research, the enzymology of lignin biodegradation has been partially determined, although there is still no mixture of enzymes available which convincingly produces delignification of wood or pulp. In other enzyme studies, the structures of a cellulase and xylanase have recently been determined.

The second section of the report analyses factors which have contributed to the success or failure of biotechnologies since 1984. As with all technologies, those that meet an industry need at a reasonable cost have been successful. Biological waste water treatment has been pushed by legislation. On the other hand, the supplier companies have played an important role in the application of enzyme treatments. Several promising applications identified in 1984 have not yet been applied. We have attempted to determine why.

The second section of the report also compares pure and applied research in those countries considered to be important in biotechnology, namely Canada, Finland, Japan, Sweden and the USA. We conclude that Canada compares well with other countries. However, since pulp and paper is such an important Canadian industry, it is imperative to maintain a leading position by encouraging both pure and applied research, and technology transfer. A number of questions addressing these tasks are suggested as topics for discussion at the forthcoming workshop.

TABLE OF CONTENTS

INTRODUCTION

HIGHLIGHTS OF PROGRESS TOWARDS APPLICATIONS OF BIOTECHNOLOGY IN THE PULP AND PAPER INDUSTRY.

1. **Progress in industrial applications.**
   a) Waste-water treatment
   b) Mill-scale bleaching trials with xylanase
   c) Mill-scale trials on dewatering in papermaking
2. **Progress in applied research.**
   a) Biological bleaching
   b) Biomechanical pulping
   c) Enzymatic pitch control
3. **Progress in fundamental research.**
   a) Lignin biodegradation
   b) Molecular mechanism of cellulase action

DISCUSSION OF FACTORS INFLUENCING THE DEVELOPMENT OF APPLICATIONS OF BIOTECHNOLOGY IN THE PULP AND PAPER INDUSTRY.

1. **Biotechnologies applied since 1984, or likely to be applied.**
   a) Anaerobic and aerobic effluent treatments
   b) Biological bleaching of kraft pulps
   c) Deresination of wood chips and pulp

2. **Biotechnologies identified in 1984, but not yet applied.**

   a) Biomechanical pulping

   b) Ligninase and biomimetic pulping

   c) Wood protection during chip storage

   d) Control of slime in pulp and paper operations

   e) Glucose via enzymic hydrolysis of primary clarifier sludge

   f) Color removal from pulp bleaching effluent

3. **Canada relative to other countries.**

4. **Technology transfer issues.**

REFERENCES

INTRODUCTION

In terms of value-added product, the forest products industry is Canada's largest primary manufacturing sector. For example in 1985 the value added by the forest products industries was $12.2 billion, nearly double the next largest sector, primary metal products. The forest products industry is also by far the largest Canadian exporter. In 1988, the net exports of Canadian forest products were $18.8 billion, three times more than the next biggest contributor, metal ores and non-ferrous metals (Anon, 1988).

The application of biotechnology in the pulp and paper industry is a logical choice since the raw material, wood, is a biological product and as such is biodegradable. Biodegradability makes wood and wood products amenable to various biological and biochemical treatments. Thus processes based on a controlled effect of wood degrading microorganisms, natural or genetically engineered, are possible. Enzymes specifically suited to modification of individual wood components exist in nature and are therefore potential candidates for industrial exploitation. Potential applications have recently been reviewed by Trotter (1990a and 1990b) and a comprehensive treatment of the subject can be found in a recent book edited by Kirk and Chang (1990).

Unlike some high technology industries, the pulp and paper industry is characterized by extremely large production volumes. Thus the pulp and paper applications of biotechnology are also likely to be on very large scale. Full scale application of microorganisms in the industry would probably represent a major fermentation business, surpassing any of the major present fermentation industries, such as alcoholic beverage production. If a successful application of enzymes were developed in the industry, it would again be a very major effort.

It was estimated by a speaker at the recent Cellucon'90 conference in Bratislava, Czechoslovakia, that if an enzyme application were adopted by the pulp and paper industry, this alone could double the present world production of enzymes. Thus the pulp and paper industry could have an enormous impact on biotechnology. In the long run, it could become a bigger money spinner for biotechnology in Canada than pharmaceuticals.

The objective of this report is to examine whether indeed the opportunities are real and if so, to help focus on what could be done to facilitate technology development and transfer. The reality of the opportunities is documented by a number of processes invented in the laboratory and shown to be technically feasible. Some of these processes are still in conceptual state and are based just on fundamental research. Other ideas, closer to industrial application, are those verified by applied research and tested on bench-top scale. The strongest arguments supporting the validity of the biotechnology opportunities are processes that have been successfully tested in a pilot-plant or full mill scale. It is encouraging to observe that more of the latter are gradually appearing. As for the second part of the objective, this report will analyze the developments of biotechnology during the last few years and try to identify the key factors which influenced the development. This analysis will also attempt to identify bottlenecks that should be removed. From this analysis, it may be possible to determine what, within the power of the government, could be done to speed up the progress of applications of biotechnology in the pulp and paper industry.

In 1984 the Science Council of Canada produced a report "Seeds of Renewal" (Anon, 1985, a,b) on the opportunities of biotechnology in the renewable resource industries including the pulp and paper industry. A manuscript report on applications of biotechnology

was issued as a background document (Jurasek and Paice, 1984). These documents will be used as a reference to determine whether progress has been made in the last few years. Revisitation of these earlier reports will also provide a learning experience hopefully telling us about the predictability (or unpredictability) of the development of this emerging technology. It will be interesting to observe whether pulp and paper biotechnology is still as exciting as it was before, where it has failed to meet expectations, and where it has already delivered some benefit to the industry.

Thus this report will first review some recent developments of the pulp and paper biotechnology, and then analyze factors that have stimulated or impeded the developments in Canada. Much of what is being said in this report is a matter of opinion and as such is open to discussion and hopefully constructive criticism.

HIGHLIGHTS OF PROGRESS TOWARDS APPLICATIONS OF BIOTECHNOLOGY IN THE PULP AND PAPER INDUSTRY

We have solicited advice in this area from researchers who we consider to be active in applied research. A letter was sent to ten people (Appendix A) and the opinions of the six respondents have been included in this section. In addition, we have recently had visits from two Japanese research groups (Kobe Steel and Kyushu University) and their results are also summarized.

1. Progress in industrial applications.

a) Waste water treatment

In the last three years, environmental issues associated with pulp and paper production have again, as in the 1970s, become of concern to the Canadian public, due mainly to the discovery of dioxins in bleached kraft pulp products and in effluents from bleach plants. Although the dioxin issue has been successfully resolved by in-plant modifications of the process as well as external treatment, attention has now shifted to other chloroorganics in bleach plant effluents, and indeed to most of the conventional environmental parameters (toxicity, BOD, etc).

Bleached Kraft Pulp Mills

Many kraft mills in Canada have operated biological secondary treatments for a number of years. Generally, these treatments are aerated lagoons with long hydraulic retention times (typically 5 - 10 days). Aerated lagoons efficiently reduce biochemical oxygen demand (BOD), and remove dissolved organic material which causes causing acute toxicity to fish. However, not all mills have enough land space to accommodate a lagoon of the required

size. It appears certain that secondary treatment will be required by legislation sometime soon. For example, in BC secondary treatment will be required by the end of 1991. This has prompted a recent spate of pilot plant studies and construction of full scale biotreatment plants. Most of these are high rate aerobic processes, various configurations of the activated sludge process. However, there is also some interest in anaerobic treatment. It is hoped that as well as eliminating BOD, these plants will reduce the discharge of organochlorine compounds as measured by the AOX parameter. There is some published evidence from Finland and Canada that this may indeed occur (Gergov et al., 1988, Cook, 1989).

Since 1984 at least seven activated sludge units have been constructed or are under construction at bleached kraft mills in Canada. Several mills have chosen the UNOX system, which uses pure oxygen for aeration and was originally developed by Union Carbide. An outline of the system is shown in Figure 1. As with all activated sludge systems, sludge recycle provides a high rate of microbial action and a low discharge of suspended solids. One advantage of the UNOX system is that, because it is a closed system, release of odorous off-gasses is minimized. This is important at locations where mills are situated close to a population centre. However, in a pilot study (Rempel et al., 1990), it was found that both air and oxygen activated sludge were equally efficient in BOD removal at the same hydraulic retention time. AOX removal was between 14 and 67%, compared to 48-65% removal in the Finnish mill study mentioned above.

The biodegradation of AOX is a complicated subject since the AOX parameter includes many different chloroorganic compounds. Mueller et al. (1977) concluded that aerobic secondary treatment would biodegrade some chlorophenols and chlorinated resin acids,

while others would be only partially removed. On the other hand, high molecular weight chlorolignin is not significantly degraded by aerobic systems (Eriksson et al., 1985). However, two approaches to chlorolignin biodegradation have been tried. One involves combined anaerobic aerobic treatment which was reported to remove up to 70% of AOX in a Finnish pilot study (Hakulinen and Salkinoja-Salonen 1982). Bacteria have recently been identified which perform anaerobic dechlorination of simple chlorinated aromatics, such as chlorobenzoate (Stevens et al., 1988). The other approach is to use an established culture of white-rot fungi in a reactor which will allow selective growth and high aeration (e.g. the Mycor process, Figure 2). White-rot fungi in the presence of readily metabolized carbon sources such as sugar will mineralize chlorolignin (Chang et al., 1987, Bourbonnais and Paice, 1987). The process has not yet been applied in a mill, probably because the feasibility of establishing and maintaining the fungus in a mill environment has not been demonstrated.

One unique approach to secondary treatment of bleach-plant effluent is practised at the Crestbrook mill in Skookumchuk, B.C. Effluent is allowed to permeate through seven limestone-lined lagoons, each filled on a daily basis. The effluent drains through the limestone to the receiving water, and pollutants are trapped on the limestone where they gradually biodegrade (Anon, 1981). This rapid infiltration method removes colour and AOX as well as BOD and other organic components.

Turning to biotreatments at kraft mills in other countries, the situation is quite variable. The Swedish industry, for example, have very few biotreatments because the kraft mills originally hoped to meet pollution standards by installation of oxygen delignification.

However, the environmental benefits which arise from biological treatment are now appreciated, and there is a move to install biological treatment systems at all mills.

In Finland, where oxygen delignification is not commonly used, biological secondary treatment is often practised in kraft mills - either aerated lagoons or activated sludge systems with fairly long hydraulic retention times. One kraft mill has been running a full-scale anaerobic system but this has recently been discontinued.

In Australia and New Zealand, several novel secondary treatments have been tried. One mill treated chlorination stage and extraction stage effluent separately. Only the C-stage AOX was effectively removed (Stuthridge et al., 1990). Pilot plant studies in New Zealand with anaerobic bacteria immobilized on granular carbon have been reported to successfully remove colour, although the long-term efficiency of the system was not established (McFarlane and Greenwood, 1986).

In the U.S.A., newer kraft mills have included aerobic secondary treatments which, as discussed previously, will efficiently remove BOD and acute toxicity. Combined physical and biological treatment might also be a possible alternative. For example, ultrafiltration will segregate high molecular weight chlorolignin and colour from more easily degraded low molecular weight organics which can be treated by conventional biotreatments (Lundahl and Mansson, 1980; Ek and Kolar, 1990). It may be possible to concentrate and burn the chlorolignin. Generally, regulations concerning AOX in the USA are only now being debated and there is less public awareness of this issue than in Europe. There may be an opportunity here for export of Canadian know-how. Bleaching technology is changing quickly in Canadian kraft mills in response to the chloroorganic issue. Already many mills

have, to a substantial extent, substituted chlorine with chlorine dioxide in the first stage of bleaching. Oxygen delignification has also been installed in several mills. Obviously, it will be necessary to determine how these process changes will influence the biotreatment. In the longer term, there is renewed interest in closed cycle concepts (such as Reeve/Rapson) which, if successful, will minimize the requirement for ex-plant biotreatment.

Newsprint, Groundwood and Specialty Pulp Mills

Whereas many bleached kraft mills in Canada have had secondary treatments for some time, this is not the case for the newsprint, groundwood, and specialty pulp mills. It appears that all mills will be required to install biological treatment to meet forthcoming regulations. At the time of writing, the regulations will require a maximum average annual discharge of 5 kg/tonne BOD_5 and of suspended solids of 7.5 kg/tonne. Acute toxicity will also be regulated. All of these regulations can probably be met with conventional biological treatments. Acute toxicity is caused mainly by extractives from wood (resin and fatty acids) and these can often be removed by aerobic treatments. However, because many different manufacturing technologies are employed, the solution to environmental problems will be very site-specific. In particular, some chemi-thermomechanical pulp (CTMP) mill effluents are proving to be difficult to detoxify consistently (Cornacchio and Hall, 1989).

CTMP effluents are highly concentrated in BOD, and especially in resin and fatty acids. This is because CTMP mills have very low water useage relative to kraft and TMP mills. BOD is approximately inversely proportional to pulp yield. Effluent temperature is typically around 60°C, and the effluent may have to be cooled prior to biotreatment. Several CTMP mills have installed pilot scale or full-scale anaerobic treatment plants. Stone-Consolidated

ran parallel anaerobic pilot plants at Bathurst, N.B. (Schneider et al, 1988), and eventually selected a combined anaerobic/aerobic system from Biothane. At least three other mills are actively involved in trials with anaerobic treatments. Usually, an aerobic polishing is required following anaerobic treatment to eliminate toxicity which persists (or is created) through the anaerobic treatment. In principle, an anaerobic plant should have lower running costs because less aeration is required and there could be an energy payback through methane combustion. In practice, sulphur contamination, which results in hydrogen sulphide generation, makes combustion of the anerobic gases less attractive.

An activated sludge system at Thorold, Ontario has been treating effluent from a recycled newsprint plant since 1981. This plant has expanded recently with the increased demand for recycled paper. Other mills of this type are in the planning stages and will inevitably lead to another waste disposal problem, namely disposal of primary and secondary sludges. Primary sludge, mainly fibres and filler, is simply the solids discharged in the effluent before biotreatment. Most mills allow this material to settle and either landfill or press and burn it. Secondary sludge is essentially the excess biomass generated by the biotreatment system. High-rate activated sludge plants produce most excess sludge. It is disposed of like primary sludge, or partially digested by aerobic or anaerobic treatment. No sludge disposal technique is ideal. Burning can generate undesirable air emissions and is often not able to generate energy due to difficulty in dewatering. Land- filling is becoming expensive due to transportation costs and concerns about groundwater contamination.

In Finland, where there are about 60 pulp and paper mills, 13 mills have activated sludge systems, out of 20 practising secondary treatment (Saunamaki, 1986). Hydraulic

retention times vary between 2 and 18 hours. Secondary sludge is either burnt (3 mills) or landfilled (7 mills, 1986 data). In Sweden, there are seven CTMP mills and four of these operate biological treatment plants. Two of these are combined anaerobic and aerobic. Several mills also detoxify their effluents by chemical sedimentation (Almemark and Frostell, 1989).

Fermentation of SSL to ethanol

Only four mills in Canada continue to produce low-yield sulfite pulp. One of these, Tembec at Temiscaming, is now constructing a fermentation ethanol plant, as well as installing extensive secondary treatment facilities (Anon, 1990a). The mill also sells lignosulfonates as a byproduct, the only mill in Canada to do this since Daishowa closed its operation in Quebec City.

Ethanol is produced by fermentation of hexoses in the spent sulfite liquor. There is also the potential to utilize pentose sugars which predominate when the mill is cooking hardwoods. Pentoses can be converted to feed yeast, furfural or, in lower yield, to ethanol (Neirinck et al., 1982). The fermentation broth containing ethanol must be distilled to concentrate and purify the product. Potential markets are in industrial uses and as a motor fuel additive. Beverage alcohol from spent sulfite liquor is not a permitted product under present Canadian law.

b) Mill-scale bleaching trials with xylanase

Several mill-scale trials with this enzyme have been performed at an Enzo-Gutzeit kraft mill in Finland (Vaheri, 1989). The principles behind the enzyme action will be discussed in detail below under applied research. The enzyme is added to brownstock prior to

chemical beaching and results in a saving of chemicals during brightness development. In a conventional chlorine-containing bleaching sequence, the Enzo-Gutzeit trials (1000 t/day) resulted in a 30% reduction of chlorine consumption, and decreased AOX in effluents and pulp. A mill-scale trial was also performed in Japan although no details are available.

There is considerable competition amongst enzyme producers to market xylanase. In Canada, ICI (Canada) is performing research and has established a patent position (Du Manoir and Dubelsten, 1989). This company (formerly CIL) has seen its market for chlorine decrease and is looking to alternatives such as xylanase. Iogen Inc., a small research organization in Ottawa, is producing the enzyme as a byproduct from cellulase production for biomass conversion. Internationally, Novo Nordisk, Cultor, Sandoz, and Gist-Brocades all have products in various stages of development. Obviously an important factor will be the enzyme cost. A 30% reduction in chlorine consumption will save about Can $6 in chemical costs. The enzyme treatment probably costs more than this at present. If the enzyme can be simply added, for example, to a high consistency storage chest, then little capital cost would be involved. Enzo-Gutzeit are hoping to use xylanase instead of oxygen delignification, which has high capital cost.

Since many mills, in Canada and elsewhere, have a limited time to reduce AOX effluent levels, a simple addition of enzyme may appear to be an attractive proposition, even if the cost is higher than conventional bleaching.

c) Mill-scale trials on dewatering in papermaking

This idea resulted from a Eureka project between Cultor and Cellulose-du-Pin in Europe. Recycled fibre which is commonly used in Europe is apparently difficult to dewater

during papermaking. The enzyme cellulase increases the rate of water drainage, as measured by S.R. index (i.e. increased freeness) . Cellulose du Pin has a patent on this (Fuentes and Robert, 1987) and claim increased paper machine speed and improved paper quality. The results of lab scale experiments and industrial trials have been reported (Pommier et al., 1989, 1990). The enzyme, known commercially as Liftase, probably degrades fines and removes fibrils from fibrillated fibres to produce faster drainage. However, the opposite effect, i.e., fibrillation, has also been reported with cellulase (Yerkes, 1968).

2) Progress in applied research.

a) Biological bleaching

i) Enhancement with xylanase

The ability of xylanase to facilitate subsequent chemical bleaching of kraft pulps which we call xylanase prebleaching was first reported by Viikari et al. (1986). Xylanase prebleaching can be combined with conventional chlorine bleaching to achieve a target brightness with lower chlorine use. This results in less chemical cost and lower organochlorine concentrations in pulp and effluent (Salkinoja-Salonen et al., 1989, Vaheri et al., 1989). Alternatively the enzyme pretreatment can be used to facilitate development of new chlorine-free chemical bleaching sequences, such as with hydrogen peroxide (Viikari, et al., 1987) or chlorine dioxide. Since 1986, other hemicellulases have been tested. Although some synergistic effect was observed with mixtures of enzymes, it was concluded that in both birch and pine pulp, the predominant effect was due to xylanase (Kantelinan et al., 1988; Viikari et al., 1990, Linko et al., 1989). Indeed, the prebleaching was observed

with highly purified xylanase (Paice et al.; 1988). On the other hand, Clark et al. (1990) found that xylanase and mannanase were equally efficient at prebleaching radiata pine kraft pulp.

How do hemicellulases aid in bleaching of kraft pulp? Bleaching typically involves delignification by chlorination and alkaline extraction, followed by brightening with chlorine dioxide. Hemicellulases may facilitate bleaching reactions by selectively hydrolysing hemicellulose in the secondary fibre wall. This would allow better access of bleaching chemicals to residual lignin or easier escape of lignin macromolecules. Alternatively, hemicelluloses covalently bound to lignin may be hydrolysed resulting in a lower molecular weight lignin-carbohydrate complex capable of faster diffusion from the fibre. These alternative schemes are outlined in Figure 3. Certainly, it is known from the work of Yllner and Enstrom (1957) that xylans initially dissolved in kraft cooking liquor can reprecipitate on and within fibres. From studies with externally added radiolabelled xylan, Clayton and Stone (1963) concluded that xylan re-adsorption on alkaline cooking of birchwood varies between 1% and 3% on pulp. It is conceivable that even more pronounced rearrangement occurs within the confines of the secondary wall where high molecular weight dissolved xylan could be redeposited during the kraft cook without leaving the fibres.

Thus, a possible explanation for xylanase action in bleaching is that the enzyme strips away xylans which physically shield residual lignin from bleaching chemicals (Fig. 3). If this were the only mechanism, then hydrolysis of galactoglucomannans should not facilitate bleaching. Experiments with pure mannanase which would answer this question have not yet been reported. However, with crude enzyme preparations, Kantelinen et al. (1988)

noted that the combined use of two enzymes - xylanase and mannanase - did not enhance lignin removal compared with xylanase alone.

One potential problem with xylanase prebleaching is the possible loss of fibre strength due to hydrolysis of cellulose by contaminating cellulase. This could occur if the pulp has to be stored for an extended period between xylanase and chemical bleaching, or if excess enzyme is inadvertently added to pulp. The cellulase content of xylanase preparations can be lowered by a variety of techniques including strain selection, cloning, or fractionation (Wong et al., 1988). These techniques must still be compatible with low-cost production.

Another potential problem could be the variation of enzyme effectiveness on pulp from different wood species. The enzyme has so far been reported to prebleach pine, black spruce, beech, mixed hardwood and aspen. Most xylanases work best at pH 5, which means that brownstock has to be well washed and then acidified. Xylanases which function in the alkaline pH range (~8) will soon be available commercially. Stability to temperature and carry-over chemicals are also important factors which must be taken into account during mill trials. Since bulk quantities of enzyme will be required if this application becomes common, it seems likely that manufacturing facilities will be developed in Canada, to minimize transportation costs.

ii) Direct bleaching with fungi

Research at Paprican has resulted in a patented process to remove lignin from kraft pulp (Paice et al., 1989). The process results in increased brightness of hardwood pulps when treated with the fungus Trametes (Coriolus) versicolor. Softwood pulps which have higher lignin contents are also delignified by the process (Reid et al., 1990), but brightness

development requires further chemical treatments with, for example, chlorine dioxide. The fungal mycelium can be recycled in bleaching by immobilization on polyurethane foam (Kirkpatrick et al., 1990). However, the bleaching process is rather slow (one day or longer), and recent research has attempted to determine the enzymology of the process in order to employ directly the enzymes produced by the fungus. One prominent enzyme secreted during bleaching is laccase, and this has been found to play a role in lignin depolymerization, at least with model compounds (Bourbonnais and Paice, 1990). Much of the bleaching process is performed by extracellular enzymes and/or other fungal metabolites, because pulp can be bleached when separated from mycelium by a porous filter (Archibald, F. Personal communication). Since there is only a low protein (enzyme) concentration in extracellular solutions during bleaching, it appears likely that enhanced bleaching can be obtained if a simple enzyme mixture can be identified and employed. In principle this mixture would be more effective than xylanase since it directly attacks lignin and requires no further chemical treatment for delignification. A joint project between Paprican and the Biotechnology Research Institute is continuing research on this process, which will be licensed royalty-free to Canadian pulp and paper companies.

In Japan, Kobé Steel has diversified into biotechnology and has research interests similar to those of Paprican. They have isolated a lignin-degrading fungus which delignifies kraft pulp and are currently designing a scaled up production facility to test the concept on a pilot-plant scale. They claim that their fungus is more selective than T. versicolor (i.e. attacks lignin more than cellulase), although the reaction conditions are different (Nishida et al., 1989).

The white-rot fungus Phanerochaete chrysosporium can delignify kraft pulp (Kirk and Yang, 1979, Tran and Chambers, 1987), but only in non-agitated cultures under nitrogen-limited conditions. These conditions are required for all ligninolytic action by the fungus. The physiology and enzymology of this process has been extensively studied, as described later in the section on fundamental research. Coincident with lignin degradation, the enzymes lignin peroxidase and manganese peroxidase are secreted by the fungus, together with the secondary metabolite veratryl alcohol (Kirk and Farrell, 1987, Gold et al., 1986). However, these enzymes mainly polymerize lignin in the absence of the fungus (Hammerli et al., 1986). The elusive goal of cell-free delignification has still not been reached. Other enzymes may be required - one such reductive enzyme is cellobiose quinone oxidoreductase (Westermark and Eriksson 1974). Or perhaps the fungal cell itself acts as a sink, driving the process towards depolymerization.

Hydrogen peroxide is required for peroxidase action and can be produced by several fungal oxidases, e.g., glyoxal oxidase, glucose oxidase, veratryl alcohol oxidase (Kersten and Kirk, 1987; Kelly and Reddy, 1986; Bourbonnais and Paice, 1988). The physiological concentration of H_2O_2 during kraft bleaching by T. versicolor is not sufficient to provide bleaching. However, we found previously that H_2O_2 from glucose oxidase can brighten mechanical pulps at alkaline pH (Paice and Jurasek, 1986).

iii) Biomimetics

If lignin peroxidase is involved in lignin depolymerization - still a debatable point - then why not use simple a model of the enzyme active site, namely a heme? This approach has been pursued by several research groups, including Repligen, UBC (Cui and Dolphin, 1990),

STFI (Pettersson et al., 1988), Crawford (Paszczynski et al., 1988) and Shimada (Habe et al., 1985 a,b). Water-soluble hemes were effective in delignification but also attacked cellulose. Heme in hemoglobin was marginally effective, while hemes in organic solvents appeared to delignify kraft pulps (Pettersson et al., 1988). However, the feasibility of this approach remains to be established.

In principle, many known pulping chemicals are biomimetic in that they loosely mimic redox processes performed by certain enzymes. For example, anthraquinone and sulphide delignify by redox cycles which are similar to the FAD cycle in glucose oxidase and CBQR. Whether chemists or microbes are more efficient depends upon your point of view!

b) Biomechanical Pulping

This idea based on the natural capacity of some fungi to remove lignin from wood rather selectively, has been patented by Eriksson et al. (1976), and the patent may well expire without an industrial exploitation. The major obstacle to industrial application is its low speed and necessity to process wood under sterile conditions. Significant advances have been made in the last few years towards making the process more efficient. A consortium of 13 companies supports research at the University of Wisconsin and the USDA Forest Products Lab. in Madison, Wisconsin, and this is the center where much of the recent progress is being made. Screening a number of fungi for their capability to influence the process of thermomechanical pulping has revealed that, for example, a 4-week pretreatment of loblolly pine chips with Ceriporiopsis subvermispora can save 68% of refining energy needed to produce pulp with the same freeness (Leatham et al.,1990). Plots of tensile index and scattering coefficient vs. yield of the pulps were used to characterize the bio-mechanical pulp

properties (Reid and Kirk, 1990). Subjected to this examination, biologically pretreated pulps resemble chemithermomechanical ones. The fungus seems to induce chemical changes in wood structure that resemble sulfonation. However while mild sulfonation of chips does not decrease refining energy, fungal pretreatment does to a substantial degree. On the negative side, the pretreatment leads to darkening of the pulp by a few points. This can be corrected by mild brightening with hydrogen peroxide.

Screening and optimization experiments with bio-mechanical pulps have revealed some interesting and unexpected findings. The energy-saving efficiencies of various fungi are not simply related to their ability to delignify wood as it was thought previously. Although the effect of the fungi is still probably mostly on lignin, it appears that the way the fungi modify lignin initially is more significant for the process than the way they remove it from wood. Screening of a number of fungi has also shown that there is not one fungal species that would be optimally suited for pretreatment of all pulps. On the contrary, different fungi should be used for treatment of different pulps. For example, while the fungus Ceriporiopsis subvermispora has shown best results with pine pulp, the fungus Phlebia brevispora performed best with aspen. Pretreatment with yet another fungus, Phlebia tremellosa, yielded best tensile index improvements on aspen pulp. Softwood vs. hardwood lignins and hemicelluloses are rather different in their structures and this probably accounts for their different responses to bio-pulping.

The economic analysis of the energy and chemical savings from biological-mechanical pulping (Reid and Kirk, 1990) is rather favorable and shows savings of US$33 per ton of pulp. Therefore there is room for rather sophisticated (and costly) technology while money

could still be made. However the industrial-size pretreatment reactors have not yet been designed and scaled up to test their technical and economic feasibility. More applied research is needed (and is underway) to develop and optimize the biological pretreatment technology. At this stage of the art, the suggestion is to use enormous rotating drums in which previously sterilized and then inoculated chips would slowly tumble for several days under controlled temperature and air exchange. If the requirement for continuous mixing and for pre-sterilization of the chips could be removed, the economy of the process could improve dramatically.

Unpublished reports from Kobe Steel Company (Anon, 1990 b) claim that their proprietary ligninolytic fungus IZU-154 does not require continuous mixing, degrades lignin faster, and saves more energy than any other microorganism known. According to the company representatives, the fungus reduced refining energy for beech wood pulp from 2,000 to 700 kWh/t while approximately doubling tensile, burst, and tear indices. However not much detail has been given and the results need independent confirmation before they are accepted by the research community.

Using a living fungus seems to be the only feasible way for biomechanical pulping at present. Enzymes from lignin degrading fungi have been isolated and probably tested in various laboratories but no significant effects on mechanical pulping have been reported.

In the context of mechanical pulps it is worth mentioning that besides lower strength relative to chemical pulps one of the main drawbacks of these pulps is their tendency gradually to darken. This brightness reversion can be partially offset by a treatment with crude enzyme solution from the fungus Pleurotus ostreatus according to Tachibana et al.

(1990).

c) Enzymatic Pitch Control

Triglyceride lipids from wood are reported to be a major cause of pitch deposition during papermaking. Pitch deposits can produce holes and breaks in paper on the paper machine. During bleaching of chemical pulps , the triglycerides are chlorinated which results in adhesive substances which stick to paper machine surfaces (Allen, 1975, 1976). The enzyme lipase has been added to pulp to hydrolyse triglycerides and thereby prevent pitch formation (Fischer and Messner, 1990). Novo Nordisk, Denmark, supplied the enzyme (Resinase) for this application.

This method has been successfully tested in long-run full-scale Jujo Paper Co. mill trials in Japan (Irie et al., 1990). Lipase from Candida cylindracea was selected from a number of available enzyme preparations. The enzyme was added at the rate of 3 ppm (w/v) to 4% pine groundwood pulp slurry. This was enough to hydrolyze 70% of the triglycerides, which in turn nearly eliminated all problems with pitch deposition and improved paper quality. The lipase was purchased at US$ 170/kg and, at the rate of addition, the enzyme cost was US$ 2.55/ton of pulp. Using the enzyme allowed a reduction in the amount of talc added to control pitch from 3 to 2% which resulted in savings of $0.70/ton of pulp. Thus the net cost was US$ 1.85/ton. Prospects for this technology are good since the cost of the enzyme is likely to decrease as mass production is started.

Another approach to the pitch prevention is to remove the resinous materials from chips prior to pulping . A fungal inoculum has been developed for this purpose (Blanchette et al., 1990) and is promoted by Repligen/Sandoz under the trade-name Cartapip. The inoculum

is applied by spraying chips as they are transported to chip piles on conveyor belts. The fungus then grows in the chip pile and after about two weeks, the average pitch content drops from 2.1% to 1.6%, as an example. Longer incubation has no deleterious effect on wood properties. On the contrary, the pretreatment apparently improves paper strength and pulp yields. Another beneficial effect claimed by the inventors is protection against blue stain which translates into brighter paper. The process, supposedly, also removes resin acids from chips. If this is true, then it should be expected that the toxicity of the waste waters would also decrease. This process is quite new and no information on it has been published yet. The claims need careful verification.

PROGRESS IN FUNDAMENTAL RESEARCH

a) Lignin Biodegradation

An understanding of how lignin is degraded by microorganisms, is fundamental for future applications of biotechnology in the pulping and bleaching. Progress in industrial applications and applied research such as biobleaching of chemical pulps, improvement of mechanical pulp and waste water treatments, will probably depend in good part on our capacity to identify and produce the enzymes responsible for lignin modification and depolymerisation from ligninolytic microorganism.

Lignin is a heterogeneous aromatic polymer found abundantly in wood and plant tissue (Sarkanen and Ludwig, 1971). It is composed of phenylpropanoid units, interconnected by stable carbon-carbon and ether linkages and contributes to the strength of plant cell walls by forming a rigid matrix surrounding the flexible cellulose microfibrils. Lignin is known to be considerably resistant to microbial degradation relative to other natural biopolymers

and undoubtedly is also a part of the living trees' natural protection system against predatory insects and animals. However, lignin can be degraded by some microorganisms, the most efficient group being the white-rot basidiomycete fungi. These fungi digest all components of the plant cell wall including cellulose, hemicellulose and lignin. Other groups of microorganisms, such as ascomycetes and fungi-imperfecti within the group of soft-rot fungi, are also known to degrade lignin to some extent. The brown-rot basidiomycetes are an interesting group of fungi which digest wood polysaccharides and cause demethylation of the aromatic methoxyl group, leaving lignin undegraded but modified. Evidence for lignin degradation by bacteria is still ambiguous. Although many bacteria are able to metabolize low-molecular weight constituents of lignin, their activity on polymeric lignin is not proved (Vicunia, 1988). A list of microorganisms implicated in lignin modification and degradation was reviewed by Crawford (1981).

Major advances in our understanding of biochemical and enzymological mechanisms of lignin biodegradation, have been made in the past six years, mostly with white-rot basidiomycetes. Earlier work indicated that lignin degradation by white-rot fungi was oxidative and non-specific, and was a result of complex reactions, such as cleavage of β-0-4 linkage and Cα-Cβ side chains bounds, ring opening reactions and demethylation (Crawford and Crawford, 1984). The lack of evidence for lignin degrading enzymes at this time, led to the suggestion that lignin was degraded by non-enzymic mechanisms involving extracellular reduced oxygen species, such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot}$), hydroxyl radical ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) (reviewed by Kirk and Shimada, 1985). Evidence for the involvement of H_2O_2 was conclusive, while no direct evidence supported

the involvement of the other free oxygen radicals.

Tien and Kirk (1983) and simultaneously Glenn et al.(1983), reported the isolation of an extracellular enzyme, produced by ligninolytic culture of Phanerochaete chrysosporium, which could cleave Cα - Cβ bonds in non-phenolic lignin model dimers. This enzyme which was first described as a dioxygenase, was later found to be H_2O_2 dependent and is now referred to as lignin peroxidase (LiP) or ligninase. Many isomers of this enzyme have been isolated and characterized (reviewed by Kirk and Farrell, 1987). They are all glycosylated proteins, with molecular weight around 42,000 and each containing an iron protoporphyrin IX prosthetic group. The general structure of the LiPs and their mechanism of action are characteristic of peroxidases in general, with the difference that LiP has a higher oxidation potential (Hammel et al. 1986). This enables the LiP to oxidize more substrates, including non-phenolic aryl alcohols such as veratryl alcohol to give veratraldehyde. This particular reaction is routinely used as an assay to measure ligninase activity. LiP is also known to catalyse oxidation of a large variety of non-phenolic lignin model dimers to yield numerous products. These reactions include benzylic alcohol oxidation, Cα - Cβ bond cleavage, β-aryl ether bond cleavage, aryl-Cα cleavage and ring-opening reactions (Kirk and Farrell, 1987). All of these reactions are consistent with a mechanism involving the initial one-electron oxidation of the substrate by the oxidized enzyme to form an aryl cation radical, followed by a series of nonenzymatic reactions to yield the final products.

Harvey et al. (1986) proposed that lignin degradation could occur via production of radical cations of veratryl alcohol which could be used as an electron transfer mediator to oxidize the lignin which is not accessible directly by the enzyme. Popp et al. have recently

returned to this theory (1990). In their first paper on LiP, Tien and Kirk (1983) showed some evidences that LiP was responsible for depolymerization of a preparation of ^{14}C-methoxylated lignin. However, Haemmerli et al. (1986) reported later that ligninase alone, with or without veratryl alcohol was not able to depolymerize lignin. It seems more likely that veratryl alcohol simply protects the enzyme from inactivation by excess of H_2O_2 (Tonon and Odier, 1988). Ligninase activity has also been detected in other white-rot fungi, including Trametes (Coriolus) versicolor (Jönsson et al. 1987), Phlebia radiata (Niku-Paavola et al. 1988), Pleurotus ostreatus and Bjerkandera adusta (Waldner et al., 1988).

Subsequently, a second peroxidase termed manganese peroxidase (MnP) was also discovered in the extracellular broth of P. chrysosporium by Kuwahara et al. in 1984. Like LiP, the Mn peroxidase is an iron heme enzyme of 46-KD, glycoslated and requires hydrogen peroxide. The uniqueness of this enzyme is that Mn II ions are essential to enzyme activity. The enzyme in the presence of H_2O_2, oxidizes Mn II to Mn III which in turn oxidizes free phenolic substrate to phenoxy radical intermediates (Glenn et al. 1987). The phenoxy radicals will in turn undergo further non-enzymic reactions, with high probability of radical coupling and quinone formation. The Mn peroxidase have also an oxidase activity against reducing compounds such as NAD(P)H, GSH and dithiothreitol with concomitant generation of H_2O_2 from molecular oxygen (Paszczynski et al., 1986). Another interesting observation on Mn peroxidase was from Forrester et al. (1988), who showed that when thiols were present, the enzyme was able to oxidize non-phenolic lignin model compounds. Wariishi et al. (1989), explained these observations by demonstrating that thiols were first oxidized by Mn III to give thiol radicals which in turn can oxidize a variety of non-

phenolic model compounds. Similar Mn peroxidases are also produced by T. versicolor (Johansson and Nyman, 1989) P. radiata and Phlebia tremellosa (Bonnarme and Jeffries, 1990).

Laccase (benzenediol: oxygen oxido-reductase) is a blue copper oxidase also known as polyphenol oxidase. This enzyme is produced abundantly by the majority of the lignin degrading white-rot fungi, and it was therefore assumed that laccase plays an important role in lignin degradation (reviewed by Reinhammer, 1984). Laccase catalyses the one-electron oxidation of phenols to phenoxy radicals with O_2 as electron acceptor. Three laccase fractions (laccase I, II and III) have been separated by ion exchange chromatography from the extracellular enzymes of T. versicolor (Morohoshi et al., 1987). It is generally agreed that laccases from different organisms are all glycoproteins containing up to six copper ions in their active sites. (Reinhammer, 1984). Like other phenol oxidizing enzymes such as MnP and ligninase, the effect of laccases on phenolic substrates depend more on the nature of the substrate than on the enzyme mechanism. Thus, radical coupling and polymerization is a major consequence of oxidation of various lignin related phenols by laccase (Kirk and Shimada, 1985) and also with lignin peroxidase (Haemmerli et al, 1986). However, recent work by Higuchi (1989) have shown that using the phenolic syringyl lignin model dimers, the laccase can cause $C\alpha$ - $C\beta$ and aryl-alkyl bond cleavages and $C\alpha$ carbonyl formation. He concluded that the reactions catalysed by laccase/O_2 and LiP/H_2O_2 are the same, but only with phenolic substrates. However, we recently showed that laccase from T. versicolor was also able to cleave $C\alpha$ - $C\beta$ bond in non-phenolic β-1 lignin model dimers and oxidize veratryl alcohol to veratraldehyde only when a recognized primary substrate was present

(Bourbonnais and Paice, 1990). Using the same conditions, the β-0-4 lignin model dimer was oxidized to give Cα oxidation products. Thus, laccases, in the presence of a mediator, have the same potential as ligninases to oxidize and cleave phenolic or nonphenolic lignin model compounds.

Another group of enzymes produced by most of the lignin degrading white-rot fungi are the H_2O_2 producing oxidases. In P. chrysosporium, many enzymes have been proposed for H_2O_2 generation, including the intracellular enzymes glucose-1-oxidase (Kelley and Reddy, 1986) and glucose-2-oxidase (Eriksson et al. 1986) which are associated with secondary metabolism of the fungus. Two extracellular H_2O_2 generating enzymes have been identified in P. chrysosporium; the Mn peroxidase which was shown to oxidize reduced substrate such as NADPH with coupled reduction of O_2 to produce H_2O_2 (Paszczynski et al. 1985) and more recently a glyoxal oxidase produced during secondary metabolism (Kersten and Kirk, 1987). Extracellular H_2O_2 producing oxidases are also present in other lignin degrading fungi including aromatic alcohol oxidase from Polystictus versicolor (Farmer et al. 1960) and veratryl alcohol oxidases from Pleurotus sajor-caju (Bourbonnais and Paice, 1988). A similar enzyme was also reported in Bjerkamdera adusta (Maheim et al. 1990).

It is difficult to determine the role of each of these oxidative enzymes in lignin biodegradation, because depolymerisation of polymeric lignins has not yet been demonstrated in vitro with any of these cell-free enzyme preparations. Furthermore, polymerization and quinone formation are the major consequences of oxidation of lignins in vitro, by the phenol oxidising enzymes; laccase, Mn peroxidase and ligninase (Haemmerli et al. 1986). Mechanisms that prevent polymerisation of lignin in vivo are still unknown.

Westermark and Eriksson (1974) suggested that the phenoxy radicals produced by oxidation of lignin fragments could be reduced back to phenol by the enzyme cellobiose: quinone oxidoreductase (CBQ'ase) preventing the reaction of radical coupling. This flavin containing enzyme was found in the extracellular liquids of P. chrysosporium and Polyporus versicolor (Westermark and Eriksson, 1974). A similar reducing role has also been proposed for veratryl alcohol oxidase in P. sajor-caju (Bourbonnais and Paice, 1989). Odier et al. (1988) found that CBQase does not prevent polymerization of phenols by ligninase or horse radish peroxidase. However, Ander et al. (1990) recently reported that CBQase plus cellobiose prevents polymerization of kraft lignin by lignin peroxidase. We also observed (unpublished results) using kraft lignin, that veratryl alcohol oxidase with veratryl alcohol will prevent and revert the polymerization of kraft lignin by laccase, and that the oxidation and reduction reactions were dependent on the oxygen availability.

Even if good progress is being made in describing the lignin degrading enzymes of white-rot fungi, the role of each of these enzymes in the overall process of lignin biodegradation is still unclear. Other enzymes or combination of enzymes and radical mediators still have to be discovered to explain fully the mechanism of lignin depolymerization. A hypothetical mechanism of lignin degradation in white-rot fungi is shown in Figure 4. We propose here that lignin is first oxidized by one-electron abstraction by laccase/O_2 or peroxidase/H_2O_2. To prevent the radical coupling and polymerization of the oxidized lignin and low-molecular weight radicals, a reductive system located outside the cell or associated with fungal membranes has to be present.

Another important field of research for future application of lignin degrading enzymes

in biotransformation processes or in the pulp and paper industry, is the large scale production of ligninolytic enzymes. Since the discovery of ligninase in 1983, some progress has been made for production of the peroxidases in P. chrysosporium using different immobilization techniques (Kirkpatrick and Palmer, 1987, Linko, 1988 and Jäger, 1990). However, relatively low yields of enzyme production and also the unstability of the ligninase still have to be overcome. Another approach is to mimic the lignin degrading peroxidases using simple metalloporphyrins. Shimada et al. (1984) first found that synthetic iron porphyrin can catalyse a variety of reactions with lignin model compounds. A series of stable water soluble porphyrin have been synthesized by Cui et al. (1990) and used to mimic lignin peroxidase reactions.

b) Molecular mechanism of cellulase action.

A previous manuscript report (Jurasek and Paice, 1984) pointed out how far behind the current enzymology the knowledge of cellulase action was lagging. At that time one could only speculate on the catalytic mechanism of this important enzyme. Very recently a paper was published by Rouvinen et al. (1990) which at least partly corrects this inequity. At the time when over two hundred various protein molecular structures have been solved, the paper describes the first three-dimensional structure of one of the cellulases, cellobiohydrolase II from Trichoderma reesei. This enzyme is known to hydrolyze crystalline cellulose by removing cellobiose units from the non-reducing end. It is known to consist of two main functional parts (domains), one that strongly binds to crystalline cellulose, but does not hydrolyze it, and another that binds de-crystallized cellulose and performs the hydrolytic cleavage. It was the catalytic domain that has been crystallized and its structure determined.

The domain has a rather unusual structure. Like some other protein molecules, its core consists of parallel extended polypeptide chains which form a barrel-like structure. Helical chains are on the molecular surface. But what is unusual is a structure attached to this core: a system of helical and coiled chains forms a tunnel which is filled with water in the resting molecule. There is also a small tube in the side wall of the tunnel. The extended cellulase chain presumably enters the tunnel and four of its terminal sugar residues occupy four corresponding binding subsites A-D which are located inside. The catalytic cleavage takes place between subsites B and C with the participation of two aspartic acid residues (175 and 221) of which one (221) is protonated and performs the general acid catalysis while the other (175) is charged and its function is to stabilize the reaction intermediate. A water molecule is needed to complete the hydrolytic reaction and it apparently enters the catalytic site via the side tube. The structure of the enzyme suggests that the cellulose chain enters the tunnel where it is held in a precise configuration. Next, two of its terminal residues are cut off and leave thorough the other tunnel opening. The new end of the cellulose molecule now has the same structure as the previous one (because of alternating orientations of glucose molecules along the cellulose chain) and moves deeper inside where the process is repeated.

The role of the non-catalytic crystalline-cellulose binding domain can only be speculated upon because its structure has not yet been unraveled. Perhaps it dislodges individual cellulose molecules from cellulose microcrystals, a process that clearly must precede the catalytic event because the active site of the cellulase molecule, a tunnel, cannot accommodate anything else than a free cellulose molecule.

Some of the endo-type cellulases show extensive sequence similarity with cellobiohydrolase II. The similarity pattern can be interpreted as showing that the endoglucanases have cores similar to exoglucanases but the substrate binding tunnel is not present. Instead, the molecule probably has an open cleft, accessible to cellulose molecules at any position along their length. This would then account for a more or less random mode of action of endoglucanases.

In addition to substantial progress towards understanding the enzyme mechanism, this fundamental research has resulted in some interesting leads for applications. For example it has been shown by Dr. Warren (private communication) that the cellulose binding domain can serve as a specific carrier to facilitate a variety of protein purifications since the domain can be easily and specifically attached to or dissociated from a cellulose column simply by changing ionic strength.

DISCUSSION OF FACTORS INFLUENCING THE DEVELOPMENT OF APPLICATIONS IN THE PULP AND PAPER INDUSTRY

The 1984 Science Council Report identified a number of opportunities for biotechnology in the Canadian pulp and paper industry, namely: wood protection during chip storage, slime control, enzymatic saccharification of waste cellulose, decolorization of bleachery effluent, biomechanical pulping, anaerobic effluent treatment, and application of ligninases.

Only a few of the opportunities have been exploited. This is partly because priorities of the industry have changed, and partly because other more promising alternatives emerged. We will first discuss those technologies that have been successfully applied, or that have emerged since the 1984 report was written and that appear likely to be applied. We will then discuss those technologies that were identified, but that have not been applied.

1) Biotechnologies applied since 1984, or likely to be applied.

a) Anaerobic and aerobic effluent treatments.

Effluent treatment has mainly been implemented in response to regulation from legislative authorities. Occasionally there is a payoff from energy or fibre recovery but this is usually incidental. Biological ex-plant treatments have been chosen over chemical treatments because they are more cost-effective for BOD removal. In combination with bio-treatment, many mills have reduced their water consumption (e.g. white water system closure), which results in energy and fibre conservation and a more concentrated effluent stream.

In some CTMP mills, effluent concentrations are sufficient to sustain anaerobic treatments. The full-scale facility at Bathurst, New Brunswick (Schneider et al. 1988) is a

case in point, and there are other examples at pilot-plants in several mills. The anaerobic process provides a high rate of BOD removal with little sludge production. There is a potential for energy recovery by combustion of methane. However, toxicity removal is only effective when aerobic polishing follows anaerobic treatment.

Kraft mills have opted almost exclusively for aerobic effluent treatments, because the bleach-plant effluents are usually very dilute. Newer installations have often employed on the activated sludge process rather than aerated lagoons due to limited land availability.

Operating experience with both activated sludge and anaerobic treatments is rather limited relative to experience with these same processes in municipal treatment facilities. There is an opportunity to optimize these plants for removal of not just BOD, but also AOX and toxicity. In the activated sludge process, there should also be some opportunity for sludge reduction through biological digestion. Aerobic digestion of sludge has been reported by Rempel et al. (1990).

Besides activated sludge, other high-rate aerobic systems have also been tried on mechanical pulp mill effluents. For example, a bio-filter design effectively reduces toxicity in a TMP effluent (Kantardjieff et al. 1990). It may be possible to establish microbial consortia in these different designs which can more effectively metabolize some of the unique effluents encountered in the various mill effluents.

b) Biological bleaching of kraft pulps.

This is a new but perhaps most promising application of biotechnology in pulp manufacturing. Environmental concerns about the possible long-term effects of chloroorganics generated during kraft pulp bleaching with chlorine have stimulated the

search for alternative technologies. Biological bleaching is one such technology. Direct bleaching with fungi requires no chemicals, other than nutrients, while pre-bleaching with xylanase permits a substantial reduction of chlorine charges. In combination with oxygen or peroxide bleaching, xylanase could provide a completely chlorine free bleaching sequence. The direct-bleaching fungal process is not yet ready for commercialization, but xylanase pre-bleaching seems to be. Indeed, as we mentioned earlier in this report, full-scale mill trials have been successfully conducted.

The application of xylanase pre-bleaching could be very simple. In some mills no modification of the existing equipment would be required, other then installation of the enzyme dispensing device. Other mills may have to adjust the pH of the pulp slurry before the enzyme addition. Thus, the practicality of the process depends largely on the availability, purity, and cost of the enzyme.

The enzyme is likely to be manufactured by a supplier outside of the mill and sold to the mill just like any other chemical. It is not likely that the mills would manufacture the enzyme on site; the fermentation is quite simple, but costly equipment and trained specialists would be required. Thus, the enzyme manufacture will probably support a specialized supplier industry. Novo Nordisk, Cultor, Sandoz, ICI and others are already poised to assume this role.

Development of pure xylanase preparations is a challenge for research. The presently commercially available bulk xylanase preparations are often contaminated with varying amounts of cellulase activity. Cellulase may attack pulp fibers and damage their viscosity and strength to an unacceptable degree, depending on the treatment conditions. This

contamination should ideally be entirely removed, rather than conditions manipulated to minimize viscosity loss. Genetic manipulation of the existing production strains, or simply screening for better production strains, could yield the desired product.

Enzyme cost will determine how successful the pre-bleaching technology will be. The current target cost of the enzyme is estimated at $5.00/ton of pulp. This figure is based to some extent on the replacement cost of chlorine. The present manufacturing cost of the enzyme is probably higher than this. Research has resulted in new hyper-producing strains and new fermentation methods. These are likely to reduce capital and operating costs of the fermentation plants and improve enzyme productivity.

c) Deresination of wood chips and pulp.

Two approaches to deresination of wood chips and pulp have been tried in mill-scale trials. The first, at Jujo Paper company in Japan, involved spraying the enzyme lipase, now manufactured by Novo Nordisk, onto groundwood pulp (Irie et al.,1990). The treatment cost only US$ 2.55 per ton of pulp and effectively prevented pitch formation. Factors which contributed to this successful application were the availability of enzyme at a reasonable price, and the simplicity of application.

The second approach, as pursued by Sandoz/Repligen with their Cartapip product, is to remove the extractives that cause pitch from wood chips prior to pulping. A full-scale trial with Cartapip, which is a fungal inoculum of a wood-staining mold, at Bear Island Paper Co. (Virginia, USA) resulted in decreased pitch (Unpublished information). In addition, the Cartapip is reported to decrease TMP refining energy and inhibit the growth of other wood-staining fungi thereby producing a brighter pulp. It is too early to say whether this product

will be commercially successful.

2) Biotechnologies identified in 1984, but not yet applied.

a) Biomechanical pulping.

The present technologies for mechanical pulp manufacture are quite efficient and have a number of advantages over chemical pulping. High yield and lower cost are the most obvious ones. Some of the problems are lower strength, high demand on electrical energy, lower brightness, brightness reversion, and toxicity of the waste waters. Several potential applications of biotechnology address these problems.

Pretreatment of wood chips for mechanical pulp, with various fungi has been shown in the laboratory to reduce refining energy and improve strength. Research at the Biopulping Consortium in Madison, Wi., may result in a biomechanical pulping process but not within the next year or two. The process is slow and very large reactors would be required to cope with the long retention times. Moreover, the process leads to some darkening of the pulp.

Pretreatment of chips with fungal inoculum, followed by incubation under non-sterile conditions in chip piles, is a less capital intensive alternative. The process promoted by Repligen/Sandoz is less effective for energy savings, but its advantages are claimed to be higher brightness, higher yield, less pitch, and less toxicity. All this is due to the fungal inoculum, which competes with wood-staining and wood-rotting fungi normally found in chip piles. Moreover, the inoculum consumes extractives responsible for pitch problems and for toxicity. There is not much information available about the process as yet, but according to unpublished reports it has already been tested on a mill-scale and is apparently coming on stream in a mill in Virginia. It would be interesting to know to what degree the process is

affected by climatic conditions and how it would function during Canadian winters. It is known, though, that chip piles generate heat, which may be sufficient to provide favorable conditions for the fungal inoculum.

b) Ligninase and biomimetic pulping.

The discovery of lignin-peroxidase (ligninase) in 1983, led to hopes that the enzyme would be applied in pulping and bleaching. The original reports claimed that the enzyme was capable of depolymerizing certain lignins, although most of the studies were with lignin model compounds. Results of pulping and bleaching experiments have been disappointing, since the enzyme has not been shown to be active against lignin in wood or pulps. Other enzymes or bio-reagents are apparently required with lignin peroxidase to produce delignification. Alone, its activity is limited to lignin model compounds and to some laboratory preparations not truly representative of the native lignin. Some researchers even question the enzyme's involvement in lignin biodegradation in nature. An alternative role could be detoxification of wood, thus helping white rot fungi survive in the presence of lignin degradation products generated by another, as yet unknown, enzyme. All this puts the commercial potential of lignin peroxidase in some doubt at this time.

Based on the belief that lignin peroxidase is the key lignin-degrading enzyme, some researchers (e.g. Paszczynski et al., 1988) attempted to mimic its action with simpler chemical catalysts. Since lignin-peroxidase is a porphyrin-containing enzyme, various types of porphyrin compounds have been tested as catalysts for lignin degradation by hydrogen peroxide. These attempts have been more successful than experiments with the enzyme itself. Several of these compounds indeed catalyze peroxide delignification. These

experiments are still in a laboratory stage and peroxide charges used in some of the experiments are too high to be considered for industrial application.

c) Wood protection during chip storage.

This potential application, described in the Science Council report, remains an open opportunity. Some progress in this area has been made by Forintek Canada Inc., Ottawa. A fungus was identified capable of protecting wood against rot in laboratory tests, but a satisfactory method of using this fungus has not yet been developed. Some laboratories outside Canada have also made progress in this field. Repligen/Sandoz developed a process for biological protection of wood chips against blue stain and rot, combined with chip deresination. According to unpublished reports, the process may be on the verge of commercialization. Another process, which uses a Trichoderma fungus for protection, has been advocated by Dr. Messner in Austria.

d) Control of slime in pulp and paper operations.

At the time when the Science Council report was written, a commercial method based on the use of enzyme, levanase had been advertised (Hatcher, 1980). To the best of our knowledge this process has not been successfully introduced into the industry. We do not know the why; it could be high cost, disappointing results, or simply ignorance. No research in this area has been conducted in Canada. Elsewhere, the use of bacteriophage to combat slime-forming microorganisms (Araki and Hosomi, 1990) and the use of glucose oxidase by Cultor has emerged. These ideas are being tested in the laboratory. In the meantime, chemical and not biological slime control agents are being used by the industry.

e) Glucose via enzymic hydrolysis of primary clarifier sludge.

The Science Council report was quite tentative about this opportunity and further business analysis was recommended. This idea was at that time being kept alive by the oil crisis, when "energy from biomass" was a particularly popular theme. The economics of producing glucose from cellulose have never been favorable, because the cellulose is inherently difficult to hydrolyze by either enzyme or acid.

f) Color removal from pulp bleaching effluent.

Fungal systems for color removal from E_1-stage bleachery effluent have not been applied. Since the Science Council report, the interest in treating bleachery effluents has escalated, but not for color removal. Instead, the main interest is in the removal of toxicity resulting in part from the presence of chlorinated organics (assayed as AOX). This matter is treated earlier in this report. Since the biosystems capable of removing color from the effluent are at least as effective in removing many of the chloroorganic components, the interest in biological treatment of E1 (and C1) stage effluents remains.

The main impediments to commercialization of the fungal treatment process are the slow growth and poor survival of fungi in competition with other microorganisms in an open fermentation system, such as a waste water treatment plant. Possible ways to overcome these impediments include the use of enzyme reactors, incorporating enzymes produced by the fungi, or the manipulation of the conditions in activated sludge treatment plants or aerated lagoons to favor growth of the AOX-removing microflora.

3) Canada relative to other countries.

Canada, Finland, Japan, Sweden, and USA (in alphabetical order) are the main players in pulp and paper biotechnology. This is understandable, because of their substantial

commitment to the pulp and paper industry in general. Japan has a large pulp and paper industry at home, and has increased the ownership of pulp and paper companies abroad, including Canada.

Canada has a strong program in pulp and paper biotechnology, centered around the joint Paprican/NRCC project on direct biological bleaching. A biological bleaching process has been invented and patented at Paprican. Progress has been made in understanding the bleaching mechanism, which is central to the problem of lignin biodegradation, and in optimizing its parameters. In spite of this, the biological process is still too slow for commercial application. Research is underway to increase the bleaching rate, by applying isolated bleaching enzymes. Canadian researchers have also made important contributions in the area of xylanase pre-bleaching invented in Finland. Paprican researchers were the first to treat pulp with the enzyme and to describe some of its effects in the manufacture of dissolving pulp (Paice and Jurasek, 1984). The first xylanase gene clone was also produced in Canada at Paprican (Bernier et al., 1983; Paice et al., 1986). Research on the application of xylanase continues at ICI Canada, and jointly at Paprican, UBC, and NRCC Biological Sciences Institute in Ottawa under the umbrella of PENCE (Protein Engineering Network of Centers of Excellence). A recently established NSERC Industrial Chair in Forest Products Biotechnology at UBC may also strengthen the pulp and paper biotechnology research in Canada.

As with biotechnology in manufacturing, Canada is well represented in research on biological effluent treatment. Active research groups are found at the NRCC, at Paprican,at the Wastewater Technology Centre in Burlington, Ontario, and at several universities.

Engineering groups and consultants are also available for full-scale application of existing technology.

Finland has been the home of some recent developments of pulp and paper biotechnology. Perhaps most significant was the discovery of bleaching enhancement by the enzyme xylanase in 1986. The principle and industrial potential of this discovery is described elsewhere in this report. The discovery was the result of many years of research in the government laboratories. Finland's VTT Biotechnical Laboratory (Helsinki), where much of the research has been directed toward the pulp and paper industry, collaborates with private industry, including some pulp and paper companies. Recently, the xylanase pre-bleaching process has been tested in full mill scale (1000 tons/day) by Enzo Gutzeit. The enzyme has been supplied by several companies, including the Finnish biotechnology company, Cultor (Vaheri et al., 1989).

In waste water treatment, Salkinoja-Salonen at the University of Helsinki has been very active in the field of chloroorganic degradation. A combination of anaerobic and aerobic processes (the Enzo-Fenox system) has been shown to remove chlorophenols from kraft bleach plant effluents. A full-scale application of the system was not considered a success, although research on the concept is continuing. Several bioremediation strategies for contaminated soils have also been developed.

Japan is well known for its commitment to high technology, including biotechnology, and some of this effort goes into pulp and paper biotechnology research. Fundamental research in the area has been pursued in Japan for many years. Practical applications are now beginning to emerge. They include chip deresination by lipase and biopulping and

biobleaching with a unique fungus IZU-154. Kobe Steel Co. apparently has a substantial team (40 people) striving to commercialize the applications of the fungus. Japan has a large biotechnology industry already and, if a pulp and paper application is developed there, the biotechnology industry will be ready to commercialize.

Sweden became well known for its work on bio-mechanical pulping pioneered by Karl-Erik Eriksson at STFI. Three years ago Dr. Eriksson left Sweden for the USA. Bio-mechanical pulping is now pursued in the USA, but curiously enough, not in Dr. Eriksson's laboratory but rather in Dr. Kirk's Institute at USDA.

At the Swedish Environmental Research Institute (IVL), Frostell and co-workers have successfully applied combined and anaerobic and aerobic systems in full scale applications for CTMP effluents. Similarly, Welander and co-workers (SCA and Lund University of Technology) have developed the Ostrand full-scale plant.

The United States made an impact on pulp and paper biotechnology research through studies on the fungus Phanerochaete chrysosporium and its enzyme lignin peroxidase co-discovered by Dr. Kirk in the USDA Forest Products laboratory and Dr. Gold at Oregon Graduate Center. The peroxidase gene was cloned by Ming Tien at the University of Pennsylvania and also by Dr. Farrell in a biotechnology company Repligen/Sandoz. A substantial research effort has been mobilized particularly by Repligen/Sandoz in an attempt to exploit the enzyme commercially but the results have been disappointing mainly because the enzyme is not effective either for bleaching or for pulping. The Repligen/Sandoz research is now concentrating on chip deresination with a fungus and production of pure xylanase for xylanase pre-bleaching. Another U.S. priority is research on bio-mechanical

pulping, supported by a consortium of private companies grouped around a Institute headed by Dr. Kirk.

International collaborative programs in pulp and paper biotechnology have been set up since 1984 and have produced some advances. Finland and France joined in a $16 million EUREKA venture, Biopulp International, on the project "Enzymatic Treatment of High Yield Pulps". Canada and Japan collaborated for two years on a project "Fundamental Studies on Biodegradation of Lignin". The United Nations Development Program continues to sponsor a pulp and paper biotechnology project in Chile with international participation, including Canada.

4) Technology Transfer Issues

As with all new technologies, biotechnology will only succeed by finding novel, cost-competitive solutions to recognized industry problems or opportunities. Until quite recently, biotechnology did not have much to offer the pulp and paper industry in terms of new technology, and industry personnel did not often approach biologists for advice on how biotechnology could be applied. Recent progress in biotechnology has changed the picture somewhat, but there is still a need to explain the potential value of biotechnology research programs to the industry. The information network, BIOFOR, sponsored by the federal government, has tried to bring about a dialogue on biotechnology between researchers in various laboratories across Canada on the one hand and the pulp and paper industry on the other. Paprican organized the first of a series of international conferences on "Biotechnology and the Pulp and Paper Industry". Two seminars on the topic were held for the Canadian pulp and paper companies at Paprican, one at the occasion of the opening of the P.R.

Gendron Biotechnology Research Center, and later another entitled "Biotechnology Day". These events were well attended by the industry and helped maintain awareness of the potential of biotechnology.

Pulp and paper biotechnology now seems to be entering the commercial arena. Continued information exchange is more important than ever if increased technology transfer to industry is to occur.

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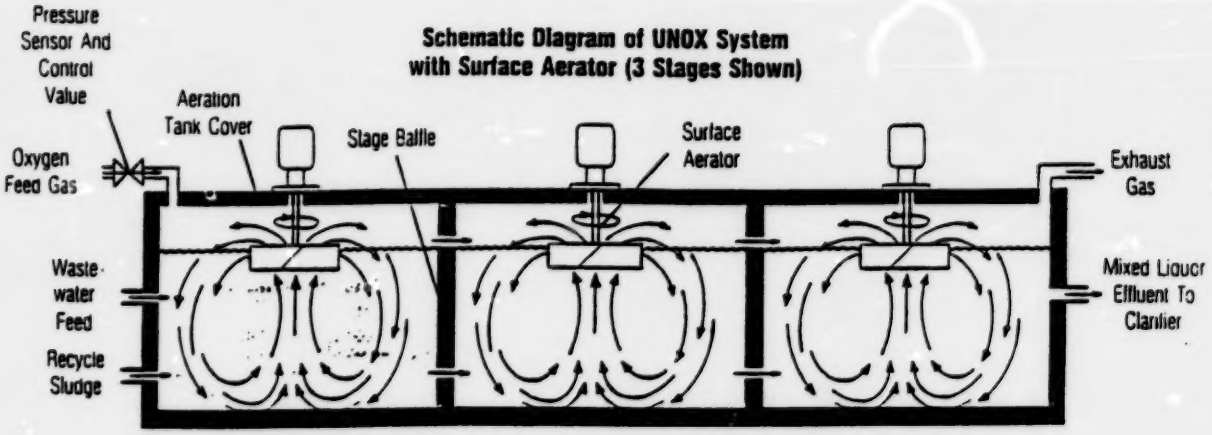

Figure 1. Schematic diagram of UNOX activated sludge system with 3-stage surface aeration design.

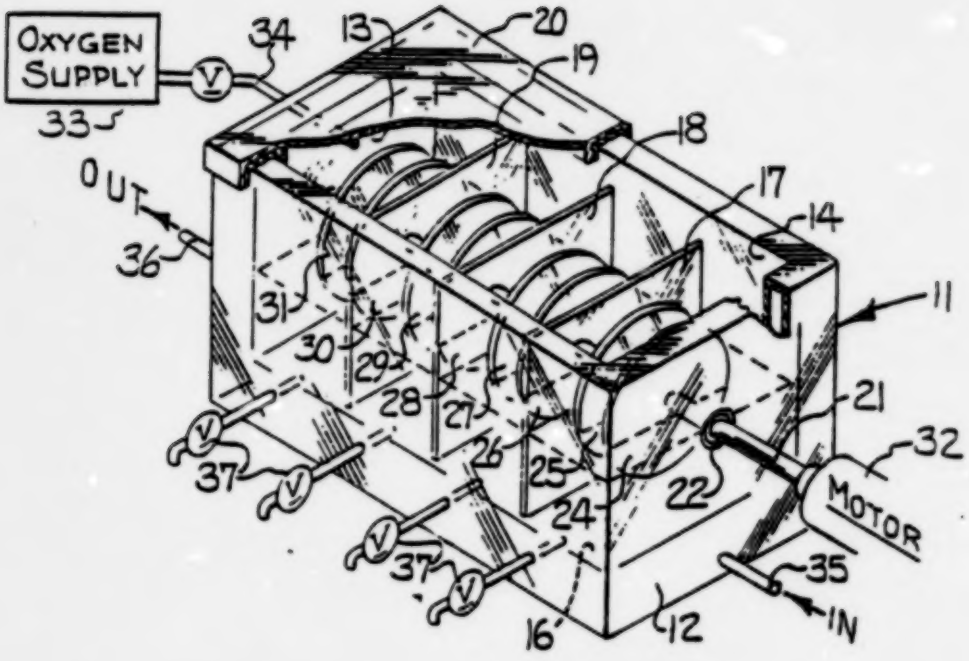

Figure 2. Rotating biological contactor which forms the basis of the Mycor process. The fungus Phanerochaete chrysosporium is established on the discs, and bleach plant effluent is passed through the lower half of the chamber.

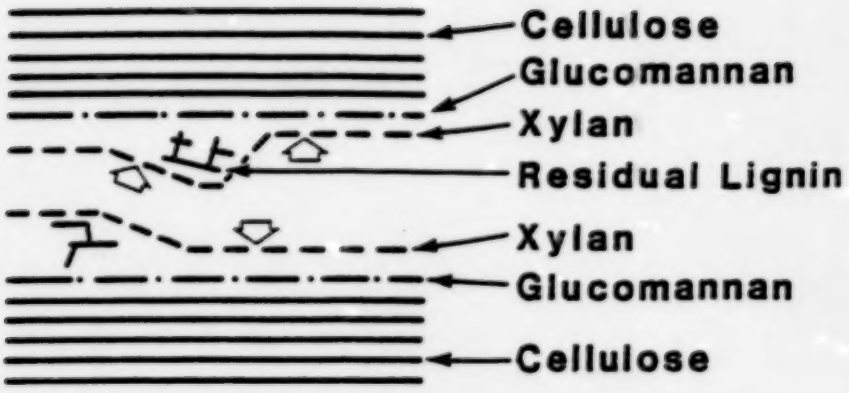

Figure 3. Proposed structure of secondary wall of unbleached softwood kraft pulp. Arrows show sites of xylanase attack. Subsequent opening up of the xylan structure results in increased accessibility to residual lignin and decreased size of lignin-carbohydrate fragments (from Reid and Paice, 1991).

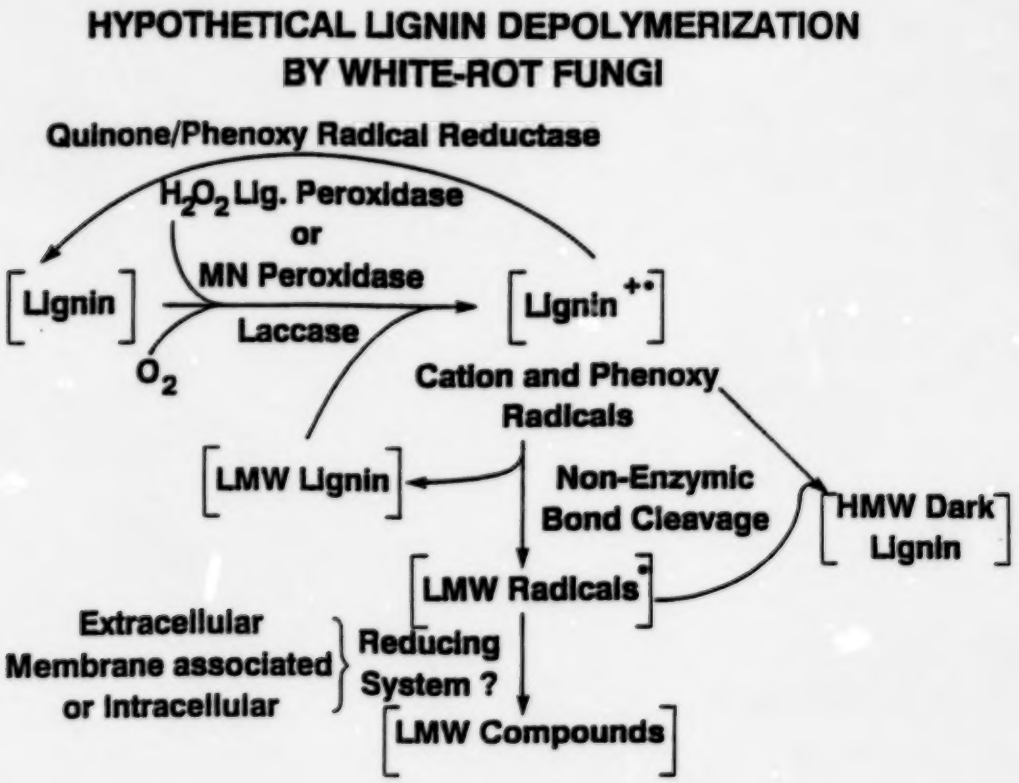

Figure 4. Hypothetical lignin biodegradation pathways.

APPENDIX A

Dear (See list attached)

We are writing a review paper for the Canadian Government on recent developments in biotechnology that may be applied to the the pulp and paper industry. The paper will be used by the Canadian National Biotechnology Advisory Committee to influence government policy in this area. The Advisory Committee is particularly interested in applications which have been successfully incorporated into mill operations, or are close to this point.

We are seeking your opinion, as a leading authority on this subject, on the likelihood of any biotechnology being applied to the industry immediately or wihtin the next two years. In order to make this review as comprehensive as possible we would also appreciate receiving any scientific or technical papers that may be too recent for us to retrieve from literature searches.

Thank you in advance for your help.

Sincerely yours,

Michael Paice
Senior Scientist

cc: L. Jurasek

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BIOTECHNOLOGY IN THE PULP AND PAPER INDUSTRY

A report prepared for the National Biotechnology Advisory Committee

by

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Pulp and Paper Research Institute of Canada

ISTC Contract No. 67-POL-0-0274

SOMMAIRE

Ce rapport a été commandé comme document de travail par le Comité consultatif national de la biotechnologie. On y procède à l'examen et à l'évaluation critique des progrès en biotechnologie appliqués à l'industrie des pâtes et papiers depuis 1984, année où le sujet avait été traité dans un rapport manuscrit du Conseil des sciences du Canada intitulé «Biotechnology in the Pulp and Paper Industry». L'objectif du rapport actuel et de l'atelier qui sera subséquemment organisé par le CCNB est de susciter une action canadienne pour aider l'industrie à mettre en œuvre les biotechnologies qui semblent les plus prometteuses.

La première partie du rapport souligne que les plus récentes découvertes en biotechnologie est divisée entre les applications à l'échelle industrielle, le développement (recherche appliquée) et la recherche fondamentale. Depuis 1984, de nombreuses autres usines font appel au traitement biologique des eaux usées, en particulier les usines de papier kraft. Il s'agit généralement d'une réaction à des lois sur la demande biologique en oxygène (DBO), la toxicité et les solides en suspension. Espérons que le traitement secondaire réduira aussi de façon substantielle les déversements organochlorés tels que mesurés selon le paramètre HOA. De nombreuses usines ont choisi le procédé de traitement par les boues activées au lieu des étangs aérés plus traditionnels, surtout en raison de contraintes de superficie. Plusieurs usines mécaniques de pâtes se sont dotées d'installations de digestion anaérobie à titre de projet pilote ou à grande échelle. Ce procédé semble particulièrement efficace dans les usines à PCTM dotées d'un effluent à DBO concentrée.

Les traitements aux enzymes facilitent le blanchiment de la pâte kraft, et ils ont réussi des tests de dépôt de poix en papeterie menés à l'étranger et à l'échelle d'une usine. Un autre traitement enzymatique à la cellulase a augmenté les taux d'essorage de la composition de fabrication recyclée au cours de la fabrication du papier. Au Canada,

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on prévoit des applications à grande échelle de certaines de ces technologies dans un prochain avenir. Des découvertes en recherche appliquée ont été observées dans le blanchiment biologique et le traitement biomécanique au moyen de champignons. En recherche fondamentale, l'enzymologie de la biodégradation de la lignine est partiellement résolue, bien qu'on ne possède encore aucun mélange d'enzymes qui produise une délignification efficace du bois ou de la pâte. Dans d'autres études sur les enzymes, on a récemment déterminé les structures d'une cellulase et de la xylanase.

La deuxième partie du rapport analyse les facteurs qui ont contribué à la réussite ou à l'échec des biotechnologies depuis 1984. Comme toutes les technologies, celles qui répondent à un besoin industriel pour un coût raisonnable ont connu la réussite. Le traitement biologique des eaux usées a été favorisé par les lois. Par contre, les fournisseurs ont joué un rôle important dans l'application des traitements enzymatiques. Plusieurs applications prometteuses identifiées en 1984 ne sont pas encore appliquées. Nous avons essayé de savoir pourquoi.

La deuxième partie du rapport comprend également la recherche pure et appliquée dans les pays dits importants en biotechnologie, notamment le Canada, la Finlande, le Japon, la Suède et les É.-U. Nous concluons que le Canada se compare avantageusement avec les autres pays. Puisque les pâtes et papiers sont une industrie importante au Canada, il est toutefois nécessaire de demeurer à la fine pointe en encourageant à la fois la recherche pure et appliquée et les transferts de technologie. Un certain nombre de questions entourant ces tâches sont suggérées comme sujets de discussion pour le futur atelier.